# Genetic engineering, production and characterisation of monomeric variants of the dimeric *Serratia marcescens* endonuclease

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Received 16 February 1998

**Abstract The *Serratia* nuclease is a non-specific endonuclease which cleaves single- and double-stranded RNA and DNA. It is a member of a large family of related endonucleases, most of which are dimers of identical subunits, with the notable exception of the *Anabaena* nuclease which is a monomer. In order to find out whether the dimer state of the *Serratia* nuclease is essential for its function we have produced variants of this nuclease which based on the crystal structure (Miller, M.D. and Krause, K.L. (1996), Protein Science 5, 24–33) were expected to be unable to dimerise. We demonstrate here that these variants, H184A, H184N, H184T and H184R, are monomers and have the same secondary structure, stability towards chemical denaturation and activity as the wild-type enzyme. This allows to conclude that the dimeric state is not essential for the catalytic function of the *Serratia* nuclease. In contrast, the S179C variant which is also a monomer shows little activity, presumably because this amino acid substitution changes the structure of the enzyme.**



*Key words:* Circular dichroism; Folding; Quaternary structure; Site-directed mutagenesis; Stability

## 1. Introduction

The extracellular *Serratia marcescens* nuclease is a non-specific endonuclease, capable of cleaving single- and double-stranded RNA and DNA with very high specific activity [2–6]. Like many other non-specific nucleases, it exhibits certain sequence preferences [3,6,7], suggesting that local structural features of the substrate influence the rate of cleavage. It requires $Mg^{2+}$ for phosphodiester bond cleavage and produces 5′-phosphorylated oligonucleotides [8–10]. Mutational analyses which were based on the crystal structure [11] and a comparison with the amino acid sequence of several related enzymes [12–14] have allowed to identify amino acid residues essential for catalysis and to suggest a mechanism of action for this enzyme [15,16] which was corroborated by kinetic analyses using various natural and chemically modified substrates [5,17].

The *Serratia* nuclease is a member of a family of non-specific nucleases found in both prokaryotic and eukaryotic organisms, some of which have been characterised in detail: in addition to the *Serratia* nuclease, NucA from *Anabaena* sp. [13,18,19], Nuc1 from *Saccharomyces cerevisiae* [12,20], endonuclease G from *Bos taurus* [14,21], and a RNA/DNA non-specific nuclease from the mold *Syncephalostrum racemosum* [22]. Others have been identified on the basis of their sequence homology to the *Serratia* nuclease, for example a homologous nuclease from *Borrelia burgdorferi* whose sequence was obtained in a genome sequencing project [23].

While the *Serratia* nuclease [24,25,1], Nuc1 [12] and endonuclease G [26] were reported to be homodimers, NucA is a monomer [19], raising the question whether the dimeric state of the *Serratia* nuclease, Nuc1 and endonuclease G is essential for the activity of these enzymes and/or whether these enzymes have to dissociate into monomers for catalysis. This is not only a question of academic interest but has a technological aspect to it, as the *Serratia* nuclease (Benzonase) is an enzyme of commercial importance, which is used to degrade nucleic acids in biochemical and pharmaceutical preparations. For this purpose efforts are being undertaken to immobilise the enzyme on solid support. This would be much easier to accomplish with an active monomeric variant.

Based on the detailed crystallographic analyses of the *Serratia* nuclease [1,27] which have clearly identified the physiological dimer interface, we have designed variants of the *Serratia* nuclease which should not be able to dimerise. We report here the genetic engineering, production and characterisation of such variants. Our results demonstrate that the dimer state of the *Serratia* nuclease is not necessary for the catalytic function of this enzyme, as monomeric variants with the same specific activity have been identified.

## 2. Materials and methods

### 2.1. In vitro mutagenesis and sequencing

In vitro mutagenesis to obtain the S179C, H184N, H184T and H184R variants was carried out using the 2-PCR method [15,28]. The H184A variant was generated applying an inverse PCR strategy [19,29]. The mutations were verified by sequencing using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase FS, on an ABI 373A DNA sequencer (Applied Biosystems) according to the supplier's protocol.

### 2.2. Overexpression and purification of Serratia nuclease and variants

The wild-type *Serratia* nuclease and the variant enzymes S179C, H184A, H184N, H184T and H184R were produced as $His_6$GlySer-tagged proteins in *E. coli* and purified as described before [25,5].

### 2.3. Gel filtration analysis

Preparative and analytical gel filtration experiments were carried out at 25°C on a Beckman Biosys 2000 HPLC system using a Merck Superformance 600-16 Fractogel EMD BioSEC (S) and a Pharmacia Superdex 75 HR 10/30 column. The protein concentration was 40 μM.

### 2.4. Analytical ultracentrifugation analysis

Analytical ultracentrifugation experiments were carried out at 20°C and 45000 $min^{-1}$ in a Beckman Optima XL-A centrifuge equipped with absorption optics and an An-50 8-place rotor. The protein con-

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*PII* S0014-5793(98)00279-8